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Dynamic Heat Capacity Changes of Laser-Irradiated Type I Collagen Films

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Background and Objective: **A common assumption made in the thermal response of biological materials due to laser irradiation is the constancy of the specific heat capacity at constant pressure, C_P. In this investigation, C_P of pure hydrated Type I collagen films is measured in time during laser irradiation.**
Study Design/Materials and Methods: **A Nd:YAG laser scanning calorimeter is developed and used to test the constant heat capacity assumption by monitoring transient, laser-induced thermal transitions in the collagen films.**
Results: **Results of preliminary studies on the irreversible, laser induced thermal denaturation of collagen with heating rates of up to 110 K/sec show a broad C_P transition that can attain large values (20 J/g K).**
Conclusion: **The magnitude of the C_P change that occurs in response to laser irradiation shows that the assumption of a constant C_P when modeling heat transport in tissues is not always valid.**

Key words: calorimetry, denaturation, fast thermal analysis, structural transitions

INTRODUCTION

To avoid nonspecific thermal damage during laser irradiation of biological tissues, heat conduction is an important physical process that must be understood prior to treatment. Many mathematical models of heat transport have been developed and used to predict and calculate temperatures in laser irradiated tissues [e.g., 1–3]. However, such models assume that the thermophysical properties (e.g., the specific heat capacity at constant pressure, C_P) of the tissue are constant during laser irradiation.

C_P can be studied using a variety of calorimetric and thermal analyses methods. Differential scanning calorimetry and quantitative thermal analysis are two methods that can be applied to the determination of C_P of a sample over a wide range of temperatures [4]. However, current thermal analysis instrumentation does not allow for the study of the fast heating that often occurs in tissues undergoing laser irradiation. In this study, the validity of the constant C_P assumption for laser-irradiated, pure hydrated Type I collagen films is investigated. Here, application of a novel laser scanning calorimetric method for determination of C_P in collagen undergoing transient laser irradiation is presented.

MATERIALS AND METHODS

Theory

The basic principle of laser scanning calorimetry is minimization of heat losses by selection of appropriate irradiation parameters (wavelength, sample thickness, and beam diameter) and restriction of the exposure time (pulse width). Heat conduction in the plane of the film (radial direction) is minimized by fixing the laser beam diameter much larger than the thickness of the sample [5, 6]. Heat conduction normal to the film (longitudinal direction) is reduced by selecting a laser irradiation wavelength so that heat generation by absorption in the sample film is nearly

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uniform throughout the thickness. Uniform heating over the thickness prevents the formation of a thermal gradient between front and back surfaces of the sample film and hence reduces any longitudinal heat flow. Because uniform heating over the thickness of the sample implies a weak attenuation and absorption of the laser radiation, only a small fraction of the incident laser energy is absorbed by the sample and thus high power lasers may be required to deliver sufficient energy to induce the desired thermal effect (e.g., glass or melting transitions in the sample) in a relatively short time period. Furthermore, short exposure times reduce thermal losses by radiation and evaporation.

When all heat losses (i.e., radial and longitudinal conduction, radiation, and convection) are insignificant during laser irradiation, a linear heating of the sample is expected unless a heat capacity change occurs, which is indicative of a molecular structural transition. When the above conditions are satisfied, C_P of the sample film is:

$$C_P(t) = \frac{1}{Ax\rho}\frac{dQ_{laser}(t)}{dT} \quad (1)$$

where dQ_{laser} (J) is the amount of heat added over a beam area A (cm^2) sufficient to cause a temperature rise dT over a film thickness x (cm), density ρ (g/cm^3), and specific heat capacity at constant pressure C_P (J/g K).

Since laser fluxes used in heating the sample did not result in ablation, *sample mass is conserved*. Consequently, density (ρ) is only a function of volume (Ax); the product $Ax\rho$ is always constant. Alternatively, density changes of the sample do not affect the C_P analysis described here as long as mass loss (e.g., ablation) does not occur.

To include time in the analysis, the following is obtained:

$$\frac{dQ_{laser}}{dT} = \frac{dQ_{laser}}{dt}\left(\frac{dT}{dt}\right)^{-1}. \quad (2)$$

We let the constant P_a, which is equal to dQ_{laser}/dt, be the energy per unit time (J/sec) *absorbed* by the sample. Using Eq. 2, C_P is rewritten as:

$$C_P(t) = \frac{P_a}{Ax\rho\frac{dT}{dt}(t)} = \frac{Q'}{\frac{dT}{dt}(t)} \quad (3)$$

where Q' equals $P_a/Ax\rho$.

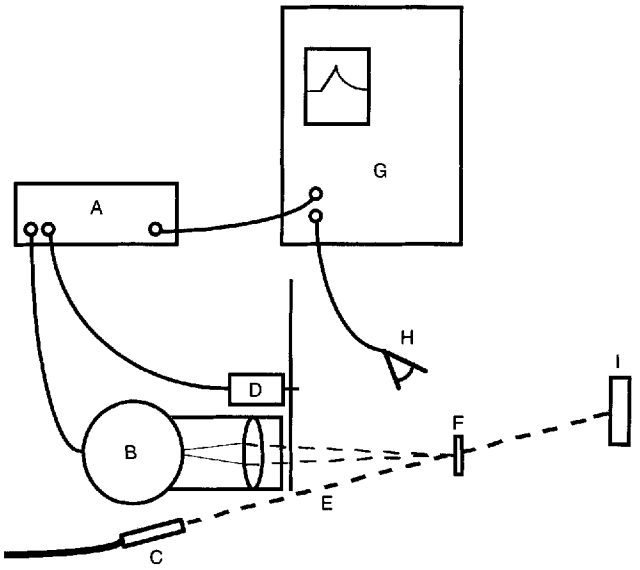

Fig. 1. Laser calorimeter. **A.** Lock-in amplifier. **B.** HgCdTe detector. **C.** Nd:YAG laser delivery fiber. **D.** Mechanical chopper. **E.** Nd:YAG laser beam. **F.** Sample. **G.** Digitizing signal analyzer. **H.** Photodetector. **I.** Beam block.

The rate of temperature change, dT/dt(t), can be determined by numerical differentiation of the measured infraradiometric temperature (e.g., using a central differences method). The constant Q' can be computed when the heat capacity and rate of temperature increase are known at some time t_o:

$$C_P(t_o)\frac{dT}{dt}(t_o) = \frac{P_a}{Ax\rho} = Q'. \quad (4)$$

We take t_o to correspond to the start of laser heating ($C_P(t_o)$ = 3.7 J/g K). Once Q' is determined, then $C_P(t)$ can be determined from Eq. 3.

Experimental Procedure

Light from a Nd:YAG laser (Cooper Laser Sonics, Laser Sonics 8000, Santa Clara, CA) was used to heat the sample at pulse widths ranging from 200 to 700 ms and powers (output from a silica multimode optical fiber) from 70–90 Watts (Fig. 1). Because absorption of Nd:YAG laser radiation (λ = 1.06 μm) by water in the film is small [7], uniform heating in the 100–150-μm-thick collagen film (Colla-Tec, Plainsboro, NJ) is attained.

Furthermore, we verified that the Nd:YAG light transmitted through the film did not change

during the course of irradiation and subsequent denaturation; hence absorbance of the sample at $\lambda = 1.06$ μm remained constant regardless of collagen denaturation. This verification was performed by measuring the transmitted Nd:YAG light with an intergrating sphere and Si photovoltaic detector (New Focus, 2001, Sunnyvale, CA) sensitive at $\lambda = 1.06$ μm.

Collagen films were hydrated in double distilled water for 2–3 hours before irradiation; care was taken to prevent exposure of the collagen films to air (<30 seconds) to minimize dehydration. The edges of the collagen film were secured to a frame to minimize mechanical movement. The laser beam diameter on the sample was kept at least ten times larger than the thickness of the film.

To monitor the transient temperature, *T(t)*, of the laser irradiated collagen, a 1 mm^2 liquid-nitrogen cooled HgCdTe infrared detector (Cincinnati Electronics, MDD-10E0-S1, Mason, OH) optically filtered for sensitivity in the 10.6–14 μm spectral region was used. Infrared emission from a 1 mm^2 area in the center of the irradiation zone was collected with a f/1 germanium lens with unit magnification conjugates. To improve the detection system signal to noise ratio, a mechanical chopper was used to modulate the incoming infrared radiation at a constant reference frequency (3,500 Hz). Synchronous detection of the modulated signal was done by a lock-in amplifier (Stanford Research Systems, SR850, Sunnyvale, CA). The infrared detection system was calibrated using a thermistor (Keithley Instrumentation, Model 8681, Cleveland, OH) attached to a resistive heater coated with high emissivity black paint (Tracor GIE, Provo, UT). Temperature varied linearly with signal amplitude over the tested temperature range of 20–70°C. Each thermal measurement was triggered using a Si photovoltaic detector (New Focus) sensitive at $\lambda = 1.06$ μm.

Calculation of the temperature derivative, dT/dt(t), was done numerically using a central differences method. Because measurement noise in the data propagated into the calculation of C_P, computed heat capacity and derivative curves were smoothed.

RESULTS AND DISCUSSION

Differences are observed in the thermal response between samples that shrunk and those that did not (Fig. 2). Shrunken samples display an obvious nonlinearity in their temperature rise, whereas nonshrunken samples had a nearly linear increase in temperature. The derivatives, dT/dt (t), of both shrunken and nonshrunken samples show a nonlinearity ($dT/dt \neq$ constant). Maximum heating rate achieved by the laser scanning calorimeter was 110 K/sec (Fig. 3). The heating (scan) rate capabilities and the lack of instrument lag make laser scanning calorimetry a suitable method of studying transient thermal events that may have short lived intermediate states.

When plotted versus time, an increase in C_P up to the transition is seen; peak values of C_P equals 20 J/gK (Fig. 4). It is obvious that C_P is not constant during Nd:YAG laser irradiation. During denaturation, the change in C_P can be large ($C_{P,max}/C_{P,o} > 4$). Consequently, the temperatures predicted by solving the bioheat equation assuming a constant C_P can be in error.

Because pure collagen films (> 99% dry mass) were used in this study, collagen in the *physiological* state is expected to be more stable against thermal denaturation. More specifically, in our experiments no proteoglycans or glycosylaminoglycans were present in the sample films to stabilize against thermal insult; consequently, lower transition temperatures (≈42°C) were measured in comparison to other studies [8]. Similarly, in network polymer crystal melting, fast heating results in reduction of the onset of melting temperature [9]. Since an irradiated film consists of a network of collagen fibrils, fast laser heating may reduce the onset transition temperature (42°C) from values measured during slow heating (45°C) [8].

The $C_P(t)$ curve for the nonshrunken sample (Fig. 4A) shows that some denaturation did occur. This finding indicates that *some* thermal damage (denaturation) to the collagen can happen without any observable global morphological changes to the fibril network. The laser scanning calorimeter may thus be more sensitive in detecting and quantifying thermal damage than histological methods currently used [10].

In general, the shape of calorimetric scans, $C_P(T)$ or $C_P(t)$, of proteins during irreversible structural transitions is not completely understood. The appearance in Figure 4D of C_p failing to attain a steady-state value toward the end of the scan (irradiation) may be due to the irreversible, nonequilibrium nature of the denaturation. However, this behavior is also indicative of incomplete collagen denaturation. A second irradiation (in same area as first irradiation) of some

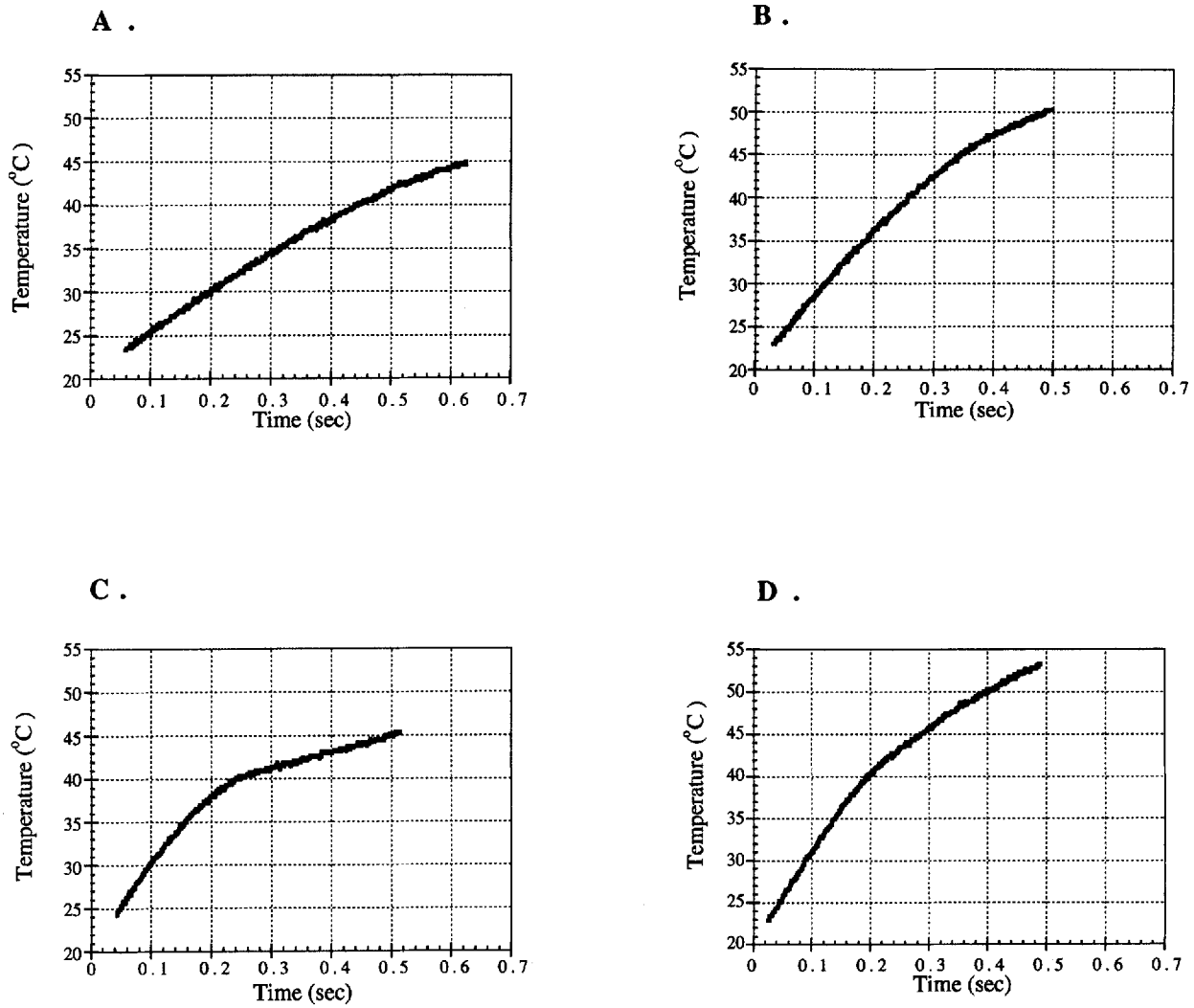

Fig. 2. Temperature vs. time curves for irradiations of collagen films. **A.** $Q' = 175$ W g^{-1}, no shrinkage observed. **B.** $Q' = 270$ W g^{-1}, shrinkage observed. **C.** $Q' = 320$ W g^{-1}, shrinkage observed. **D.** $Q' = 523$ W g^{-1}, shrinkage observed.

shrunken samples revealed a second yet smaller transition in $C_P(t)$. This smaller, secondary transition indicates that native collagen was present in the irradiation zone following the first exposure, i.e., denaturation was incomplete during the first exposure that caused shrinkage.

If the heat capacity curves shown in Figure 4 are extrapolated to longer irradiation times, we see that the transition is represented by a very broad curve. We expect a broad transition corresponding to nonequilibrium unfolding of large proteins because different parts of the molecule may denature at different times (temperatures) [11]. Furthermore, large protein molecules such as collagen have thermodynamically and structurally cooperative domains that have different stabilities [11], and thus the thermal transition of collagen is expected to be inherently broad during fast denaturation. The broad transitions and the observation of incomplete denaturation even after shrinkage may also be indicative of collagen molecules with different stabilities within the fibril network. Such a hypothesis agrees with fibril structural theory [12]. Thus, we theorize the broad transition (in time and temperature) observed in the denaturation of the collagen fibril network in this study may be explained by different thermodynamic stabilties of components of an individual collagen molecule (i.e., the cooperative domains) and also the different thermodynamic

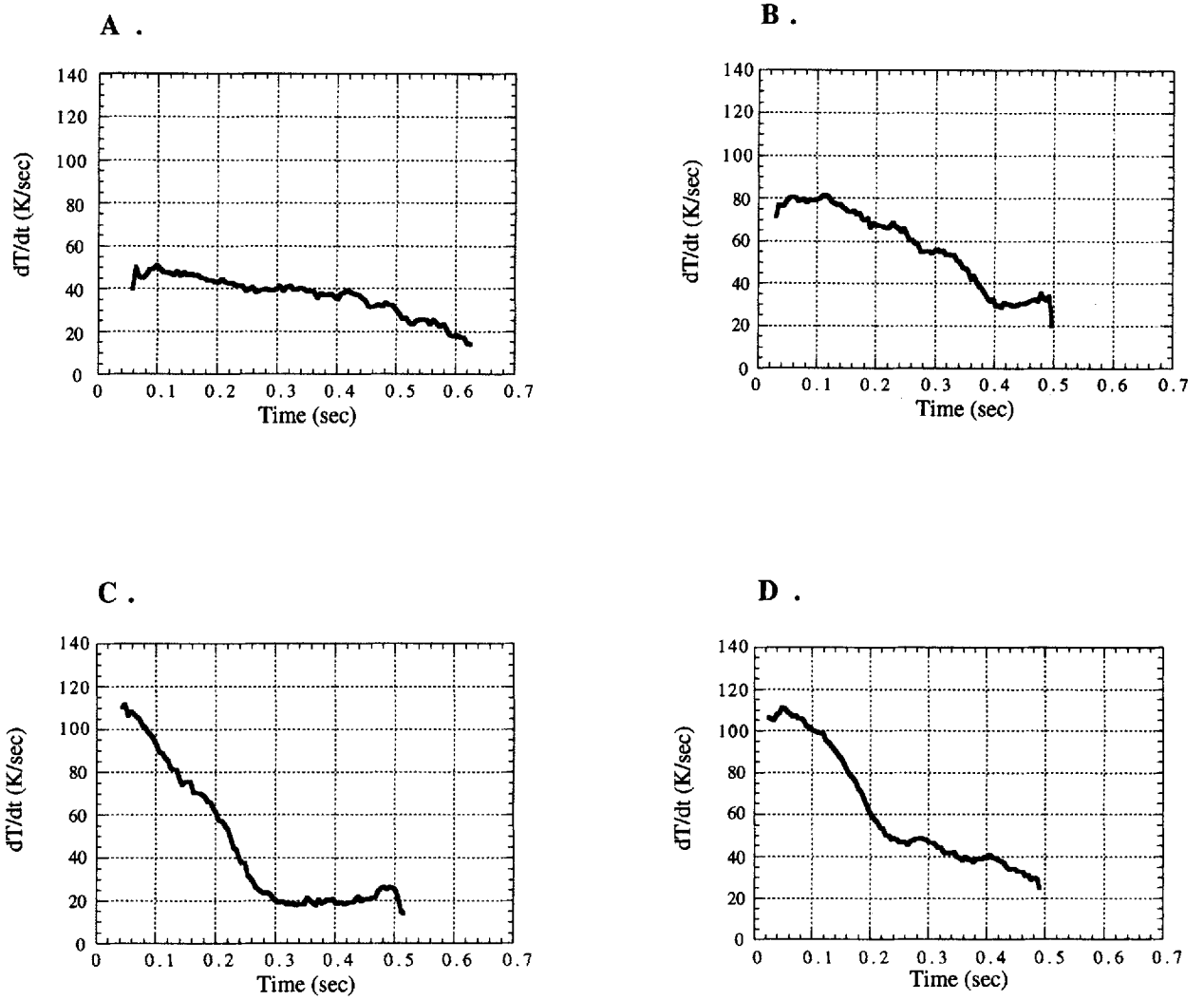

Fig. 3. Corresponding derivatives of above T vs. t data.

stabilties of *distinct* collagen molecules (i.e., collagen molecules located in different areas of the fibril).

CONCLUSION

The laser scanning calorimeter described here provides a method of determining C_P of thin films. The determination of C_P can allow subsequent studies of any structural transitions (e.g., denaturation) that occur. The results presented show that the assumption of a constant C_P used in mathematical models of heat transport in laser irradiated tissues is not always valid and in some cases yields erroneous results.

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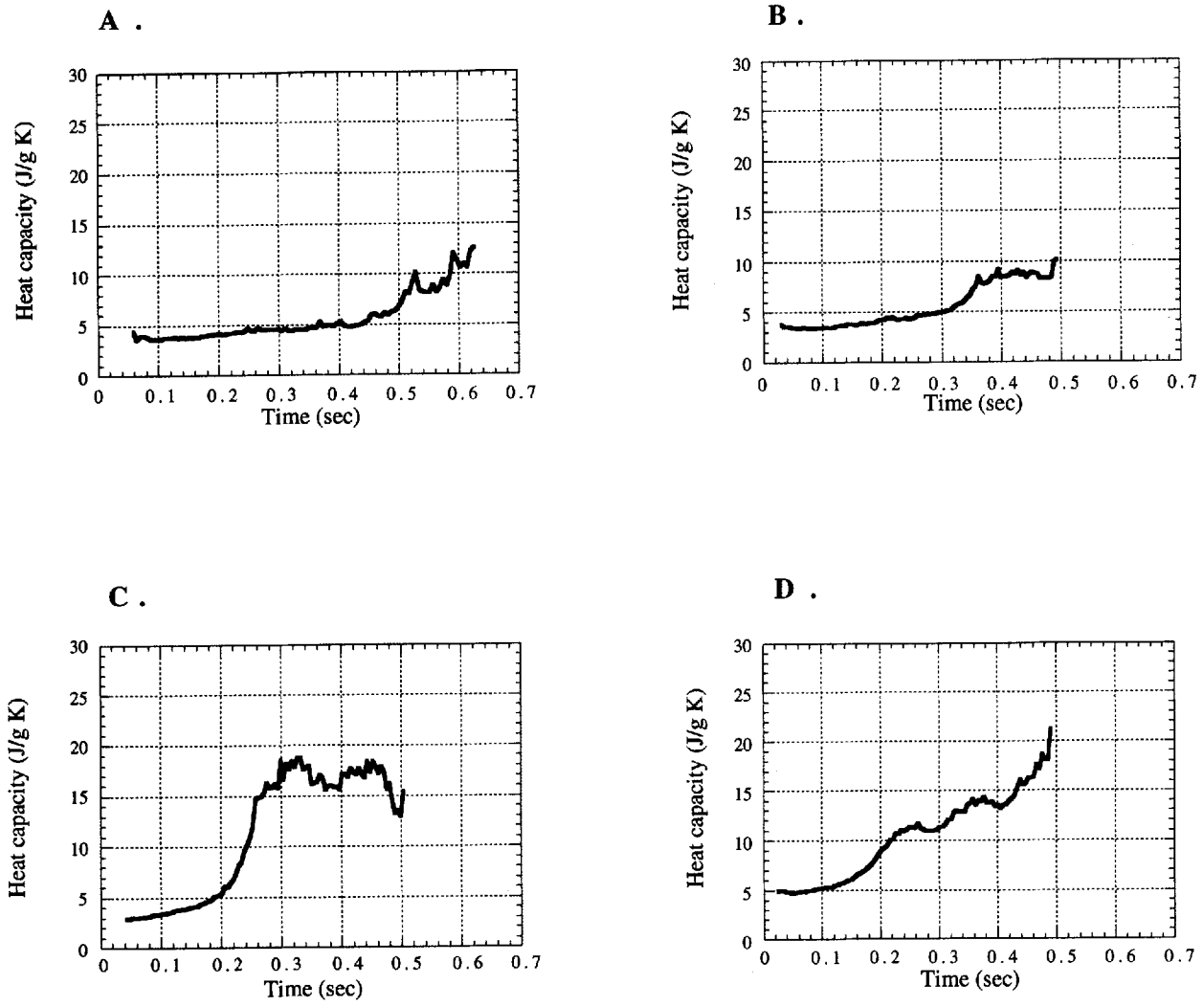

Fig. 4. Corresponding C_P vs. t data.

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